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Role of AIM in *Corynebacterium*-induced granuloma formation in mice

Kazuhisa Kuwata*[1], Hisami Watanabe[2], Takashi Yamamoto[1], Toru Miyazaki[3] and Makoto Naito[1]

Address: [1]Department of Cellular Function, Division of Cellular and Molecular Pathology, Niigata University Graduate School of Medical and Dental Sciences, 1 Asahimachi-dori, Niigata 951-8510 Japan, [2]Division of Cellular and Molecular Immunology, Center of Molecular Biosciences, University of the Ryukyus, Nishihara, Okinawa, Japan and [3]Center for Immunology, The University of Texas Southwestern Medical Center, Dallas, Texas, USA

Email: Kazuhisa Kuwata* - JZF00521@nifty.com; Hisami Watanabe - watanabe@comb.u-ryukyu.ac.jp; Takashi Yamamoto - ty@med.niigata-u.ac.jp; Toru Miyazaki - toru.miyazaki@UTSouthwestern.edu; Makoto Naito - mnaito@med.niigata-u.ac.jp

* Corresponding author

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Introduction

Apoptosis inhibitor expressed by macrophages (AIM) is a murine macrophage-specific protein and belongs to the macrophage scavenger receptor cysteine-rich domain superfamily. AIM has been introduced as the inducer of resistance to thymocyte apoptosis [1]. Because apoptosis of inflammatory cells plays a pivotal role in inflammation [2], we have applied a mouse model to address potential involvement of AIM in the process of granulomatous inflammation *in vivo*.

Methods

Animals

Mice deficient in AIM (AIM-/-) were generated by disruption exon 3 of the AIM gene (1). AIM-/- and wild-type (AIM+/+) mice were used. Heat-killed *Corynebacterium parvum* (*C. parvum*), 0.5 mg, was injected into the tail vein. All mice were killed under diethyl ether anesthesia at various time intervals after injection.

Histology

Formaldehyde-fixed and paraffin embedded livers were sectioned and stained with hematoxylin and eosin for light microscopy.

Flow Cytometric Analysis and Detection of Apoptosis

The surface phenotype of leukocytes obtained from livers was analyzed using fluorescein isothiocyanate- (FITC), phycoerythrin-, or biotin-conjugated anti-CD3 and anti-NK1.1 monoclonal antibodies in conjunction with a two- or three-color immunofluorescence test. To determine the percentage of cells undergoing apoptosis, FITC-labeled Annexin-V was used.

Results

The number of granulomas and the area size per granuloma in AIM-/- mice were larger than those in AIM+/+ mice (Figure 1). By flow cytometric analysis, there were numerical increases of conventional T cells, natural killer (NK) and NKT cells after *C. parvum* injection in the liver of both types of mice. After day 7 the numbers of NKT cells in AIM+/+ mice remained at high levels, but there was a rapid decrease of those in AIM-/- mice (Figure 2). By apoptosis detection by Annexin V, larger numbers of intrahepatic NKT cells and conventional T cells underwent apoptosis in the AIM-/- mice than in AIM+/+ mice (data not shown).

Discussion

NKT cells play a primary role in the granulomatous response of mice [3] and are associated with resistance to infection against various pathogens [4]. The present study demonstrated the poor repopulation of NKT cells in the middle and late stages of granuloma formation in AIM-/- mice. We have also observed that apoptosis of NKT and T cells after *C. parvum* injection was more prominent in

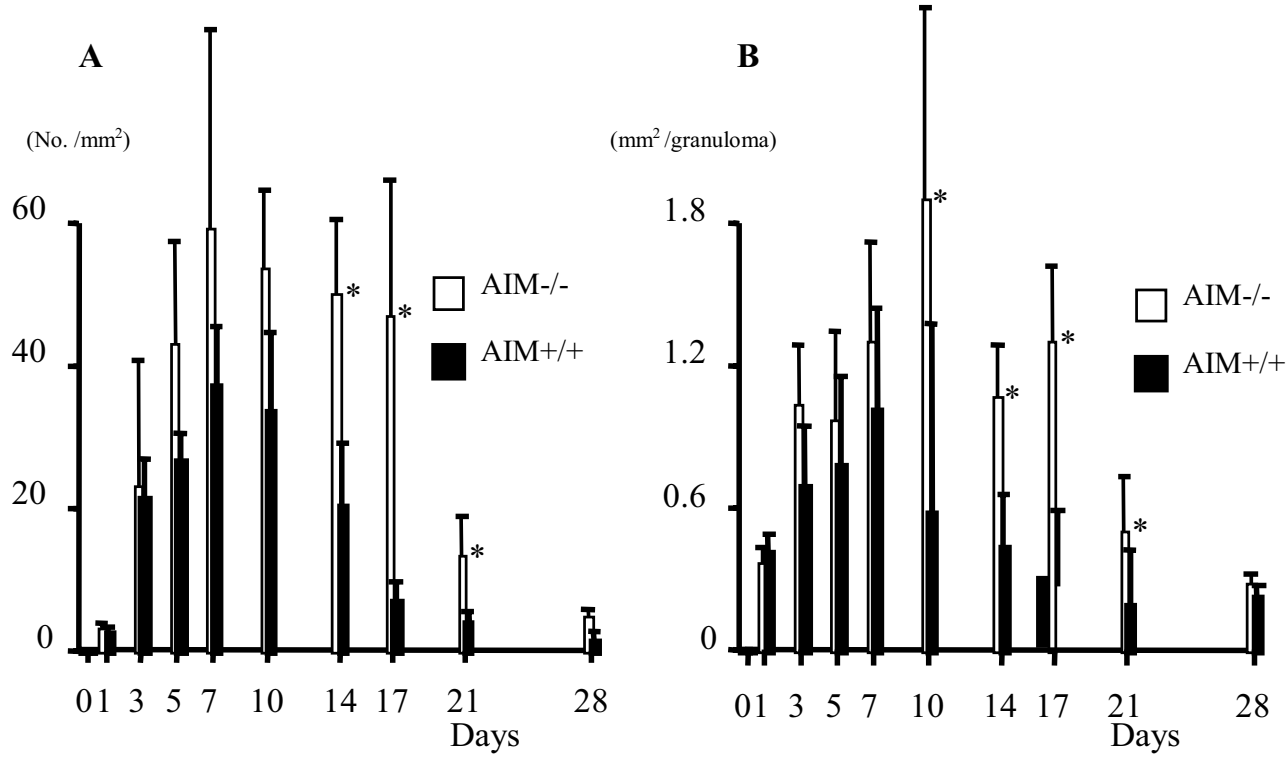

Figure 1
The number of granulomas (A) and area size per granuloma (B) in the livers of AIM-/- and AIM+/+ mice after *C. parvum* injection. AIM-/- mice developed larger numbers of granulomas than AIM+/+ mice. Mean ± SD of five mice. *, P < 0.05.

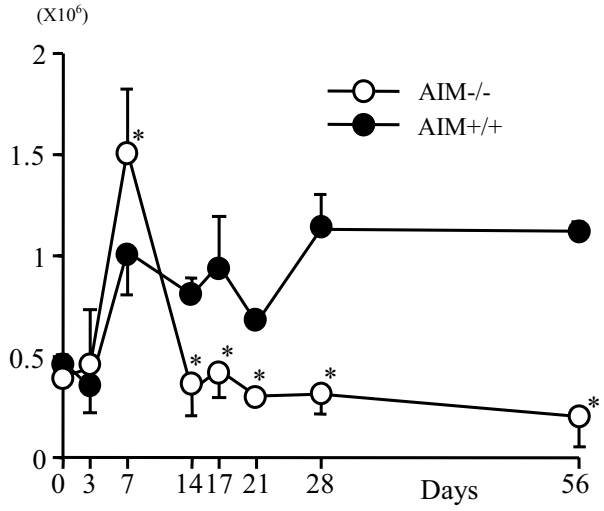

Figure 2
Absolute numbers of NKT cells in the liver of AIM-/- and AIM+/+ mice after *C. parvum* injection. The numbers of NKT cells in AIM-/- mice were significantly smaller than in AIM+/+ mice from day 14 after *C. parvum* injection. Mean ± SD of five mice. *, P < 0.05.

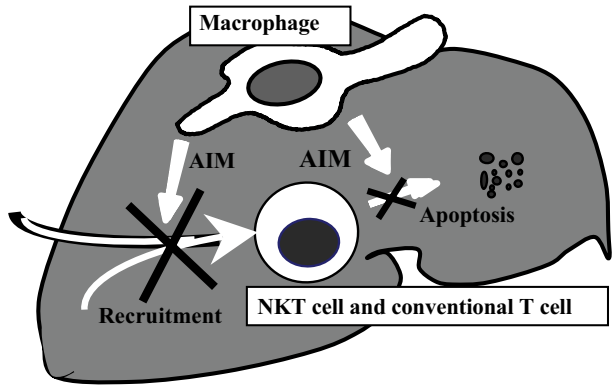

Figure 3
AIM regulates NKT and T cell apoptosis and recruitment for the formation and resorption of hepatic granulomas.

AIM-/- mice than in AIM+/+ mice. These findings suggest that AIM regulates NKT and T cell apoptosis and recruitment and plays an important role in granuloma formation (Figure 3).

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